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14. ABSTRACT
The successful treatment of prostate cancer requires detection of the disease at early stages. Currently the early diagnosis of prostate cancer largely depends on the detection of prostate-specific antigen (PSA) in circulation. However, PSA can only precisely detect 40% of prostate cancer and is not specific for the occurrence of prostate cancer. We reasoned that the success and accuracy in early diagnosis of prostate cancer may be significantly improved if a panel of prostate cancer-specific markers can be identified and used in combination for detecting early stage of prostate cancer. In the first year of the funding period, we constructed cDNA library in our pTRAP1 retroviral plasmid using RNA isolated from human prostate tumor samples. In the second year, we generated human prostate tumor cDNA library in which the signal peptides are enriched. In the third year of this funding, we screened our generated prostrate tumor library and identified 10 either secreted or cell surface proteins overexpressed in prostrate tumors. Currently, we are in the process to validate our findings and hope using these proteins as early diagnosis biomarkers for prostate cancer.

15. SUBJECT TERMS
diagnosis marker, secretion protein, early stage prostate cancer

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Introduction

The successful treatment of prostate cancer requires detection of the disease at early stages. Currently the early diagnosis of prostate cancer largely depends on the detection of prostate-specific antigen (PSA) in circulation. However, PSA can only precisely detect 40% of prostate cancer and is not specific for the occurrence of prostate cancer. We reasoned that the success and accuracy in early diagnosis of prostate cancer may be significantly improved if a panel of prostate cancer-specific markers can be identified and used in combination for detecting the early stage of prostate cancer. Our proposal aims to identify a panel of secretion proteins overproduced in early stage prostate tumors. We believe that some of these proteins can be potential candidate biomarkers for the early diagnosis of prostate cancer. Through subsequent studies that are beyond the scope of this proposal, these proteins can be further analyzed for their value as early diagnostic markers for breast cancer.

Body

The goals of our research in the final year of this funding were to 1) purify early stage tumor cells and adjacent normal cells from prostate tumor samples of 20 independent patients (months 25-27) and generate normal and tumor cDNA probes for miroarrays (months 28-29); 2) hybridize probes to microarrays of the trafficked protein library (months 30-32); 3) analyze differential expression data generated from microarrays (months 33-35); and 4) identify the clones that are overexpressed in early stage prostate tumors (month 36). The details are described in the following.

1) All prostate samples were obtained from 20 independent patients of 54-74 year olds at the Tianjin Institute of Urology, who were undergoing open prostatectomy. Harvested tissues were frozen in nitrogen and stored at -80^{0}C until use. All of the used samples were cut and analyzed by a pathologist to ensure that the samples were predominant epithelial cells (normal control samples) or carcinoma cells (cancer samples).

2) Total RNA was isolated using Trizol reagent and its integrity was confirmed by denaturing agarose gel electrophoresis. Synthesis and labeling of cDNA were achieved by direct incorporation of Cy3-dUTP in a reverse transcription reaction using total RNA, oligo-dT and Superscript III reverse transcriptase. The Cy3-labeled cDNA were resuspended in 20-μl hybridization buffer and added to our previously developed traffic protein library microarray (accomplished in the second year of this funding). Hybridization was carried out for 14 hrs at 42^{0}C inside a hybridization cassette (done in Scripps Microarray Core Facility).

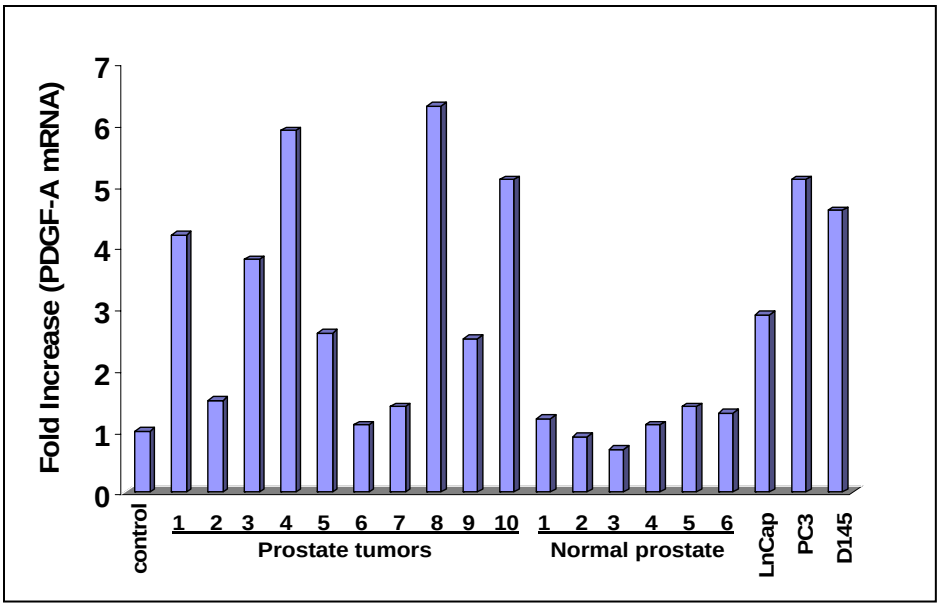
3) The hybridized slides were scanned with a ScanArray Express scanner and scanned image files analyzed with QuantArray software (Perkin-Elmer Corp.). Statistical analysis was performed using two-tailed *t* test. Only upregulated genes (exhibiting at least 2-fold upregulation) with a significant difference ($P<0.05$) between cancer and normal were selected and listed below.

Known secreted or cell surface proteins

Accession No.	Gene Name	Average Fold Change
M99487	Prostate-specific membrane antigen (PSMA)	8.2
U43318	Putative transmembrane receptor (frizzle 5)	5.1
X06374	Platelet-derived growth factor A (PDGF-A)	3.8
K03195	Glucose transporter (HepG2)	3.2
D10202	platelet-activating factor receptor	2.6
M11233	Cathepsin D	2.5
M32977	Vascular endothelial growth factor (VEGF)	2.5
NM_201636	Thromboxane A-2 receptor	2.2
M62505	C5a anaphylatoxin receptor	2.0
M91211	Receptor for advanced glycation end product (RAGE)	2.0

4) Our ultimate goal is to identify secreted or cell surface proteins that are overexpressed in early stage prostate tumors. We noticed that many of the secreted and cell surface proteins identified during analysis of the traffic protein library had been reported to be upregulated in human prostate cancer. They include PMSA, PDGF-A, cathepsin D, VEGF and RAGE.

To confirm that these identified proteins were overexpressed in early stage human prostate tumors, we compared the PDGF-A mRNA levels in human prostate tumor tissues and prostate cancer cell lines by Real-time RT-PCR (Figure). PDGF-A mRNA was upregulated in 7 out of 10 prostate tumor tissues (number 1, 3, 4, 6, 8, 9 and 10) and all three prostate cancer cell lines (LnCap, PC3 and D145) in comparison with commercially available primary prostate epithelial cells. In contrast, no increase of PDGF-A expression can be detected in any of 6 normal prostate samples (Figure). These results that our screening approach is very likely to identify proteins overexpressed in early stage prostate cancer. We are currently in the process of confirming all these identified proteins.



Key Research Accomplishment

We have identified 10 secreted/cell surface proteins overexpressed in early stage prostate tumors.

Reportable Outcomes

We have identified 10 secreted/cell surface proteins that can potentially be used as a diagnosis marker for early stage prostate cancer.

Conclusions

We have identified 10 secreted/cell surface proteins overexpressed in early stage prostate tumors. We are currently in the process of validating the results from our microarray study by real-time PCR and immunoblotting.

Both real-time PCR and immunoblotting are currently

References

None

Appendices

N/A